IQP-43-DSA-0406
IQP-43-DSA-0716

TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirement for the

Degree of Bachelor of Science

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March 14, 2005

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ABSTRACT

A transgenic animal contains genes its species does not normally have. This new technology allows animals to have a wide range of medical and research uses. The purpose of this IQP was to examine this technology and its impact on society. Areas addressed included: transgenic technology description, animal construction, classification, examples, ethical and legal concerns. This project found that with careful consideration transgenic animals can be made with little or no suffering for the animals, while providing a great deal of potential gain for humanity.

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EXECUTIVE SUMMARY

Transgenic animals are animals that have genes inserted into their DNA not normally found in their species. There are two main methods to make these animals, methods that manipulate the male pronucleus, and those that involve embryonic stem cells. Each method has advantages and disadvantages which are considered with each transgenic experiment to be performed. Transgenic animals are often made to express a protein or proteins not normally present in that animal. Since genes encode proteins, adding or removing genes can alter the proteins the animal produces. An animal's DNA can be altered to make it susceptible to a human disease its species normally could not contract so these animals can be used as model systems for human diseases and provide a system for testing new treatments and medications that would simply not be possible with human subjects. Transgenic animals have been made that serve as disease models for Alzheimer's, Sickle Cell Anemia, and some cancers.

Transpharming involves the use of a genetically modified animal to produce large amounts of a useful protein, like human growth factor, clotting factors and clot busters, usually producing the desired protein in their milk, where it can be harvested easily. For example, transgenic goats have even been made that can produce spider silk proteins in their milk for use in the biomaterials industry, or alpha-1-antitrypsin as an emphysema drug (the only transpharmed drug currently approved by the FDA).

Xenotransplantation involves animals modified to produce organs that can be transplanted into humans minimizing immunorejection. It is another long term goal of working with transgenic animals. There has been much work to develop a transgenic pig that can grow a heart suitable for transplantation into humans. Xenoplanters have the potential to save the lives

of countless people who die awaiting organ transplants, but we must pay special attention to the possibility of viral contamination of the organs.

Transgenic animals have also been made to serve as scientific models for research, and broaden human understanding. By over or under expressing certain proteins, much can be learned about the role of specific proteins on how different biological systems function and develop. Many human disorders and diseases would be better modeled on transgenic primates than transgenic mice, but the scientific community needs to first understand more about making higher order transgenic animals.

Ethics have long played a role in the biological scientific community, and they continue to play a role concerning transgenic animals. Simply because the technology to make an animal exists does not justify its creation. Current protocols require very careful consideration before any animal is created. If the benefits to society are great and there is no apparent suffering in the animal, as with Alzheimer's mouse or transpharmers, then it should be made. Conversely, if there is little benefit and the animal suffers greatly, as with Superpig, then the animal should not be made. In the latter case, biologists were the first to voluntarily impose a moritorium on performing such experiments with growth hormone. With cases where there is great benefits but also suffering for the animal, a balance must be found between minimizing the potential suffering of the animal and maximizing the potential benefit to humanity, only then should a transgenic animal be created.

With the creation of transgenic animals came the question "should animals be patented?" Legal concerns inevitably followed the ethical concerns with making transgenic animals. The process of making these animals can be very timely and expensive, and companies want to protect their assets. In the process of issuing the patent to Harvard University for the

Oncomouse, the United States became the first country to issue a patent for an animal, arguing the procedure used to create oncomouse was useful, novel, and not obvious based on prior art.

A storm of controversy followed. Several animal rights groups including WSPA (World Society for the Protection of Animals), PETA (People for the Ethical Treatment of Animals), and SACA (Students Against Cruelty to Animals) oppose the patenting of animals (Subject…2004). All of these organizations believe that the patent clearly violates the animal's right to life and to its own body. In fact, the oncomouse patent was eventually denied by Canada's Supreme Court, arguing patent laws can not apply to animals. The controversy surrounding patenting transgenic animals will no doubt not end any time soon.

The technology to create transgenic animals opened new worlds to the scientific community. This technology will enable scientists to find new disease treatments, new ways of making medication, and even whole new ways of looking at organ transplantation. With this technology comes a myriad of ethical and legal concerns that must be considered with care. It is the opinion of the authors of this IQP that transgenic technology used legally and ethically can greatly improve the quality of life for humanity, but oversight committees must continually be vigilant to enforce tight controls over ensuring mimimal animal suffering.

PROJECT OBJECTIVE

The objective of this IQP was to investigate the controversial new topic of transgenic animals, present accurate up-to-date information concerning the growing field of transgenic research, and to investigate the impact of this new technology on society by describing its ethical and legal issues. This new and controversial field has presented many new ethical and legal questions to humanity. In this IQP, the ethical implications of creating a transgenic animal will be thoroughly discussed, and the legal concerns that go along with these implications will be revealed, highlighting both the positive and negative components of transgenic research. Attempts will be made to present both sides of the moral, ethical, and legal controversies that arise with the creation of transgenic animals, then the author's conclusions will be presented to help determine whether the positive reasons for creating transgenic animals outweigh the negatives when careful procedural methods are used, and proper considerations are given to the animal's health.

Chapter 1: Transgenic Animal Description and Construction

In 1973, two University of California biologists managed to isolate and recombine the genetic material of two different organisms which would never breed in nature (Gene Safari, 2003). Thus, a new era in science began and soon scientists successfully created the first transgenic animal in the form of a mouse. A transgenic animal is defined as an animal that carries a foreign gene which has been intentionally inserted into its genome in order to cause it to exhibit traits or characteristics which are not natural to that animal (Transgenic..2003).

The foreign gene, also known as the transgene, to be used in creating the transgenic animal is constructed through a process known as recombinant DNA methodology. In this process, the transgene is inserted into a vector (Figure – 1) which allows it to be amplified to high copy numbers. The vector also contains a promoter which allows the inserted foreign DNA to be expressed by the cells of the host animal.

Figure-1: Diagram of the Cloning of a Transgene.
The transgene (pink) is inserted into a plasmid or viral vector (brown), downstream of a promoter (blue) used to control expression. This figure was taken from (Transgenic Animals, 2003).

Two Main Approaches to Transgenic Constructions

There are two main approaches to creating a transgenic animal. These approaches include either manipulating an oocyte or zygote which is then re-implanted into a foster mother, or the manipulation of embryonic stem cells which are grown to the blastocyst stage and then re-implanted.

The Pronucleus Method

The first approach, also the oldest and most common, is known as the Pronucleus Method. It is performed by first using recombinant DNA methods to clone the transgene of interest. Once this has been completed, freshly fertilized eggs are harvested before the sperm head becomes a pronucleus (Figure-2). From here the DNA is injected directly into the male pronucleus through microinjection (lower portion Figure-2, and also Figure-3).

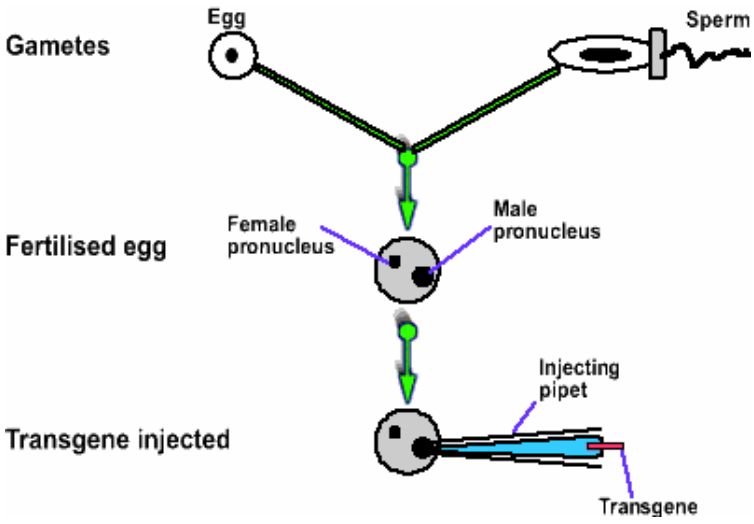

Figure-2: Diagram of the Formation of the Male Pronucleus.
In vitro fertilization is used to create a fertilized egg (diagram center), whose male pronucleus (large black sphere) is used for microinjection of the transgene solution (turquoise). This figure was taken from (Transgenic Animals, 2003).

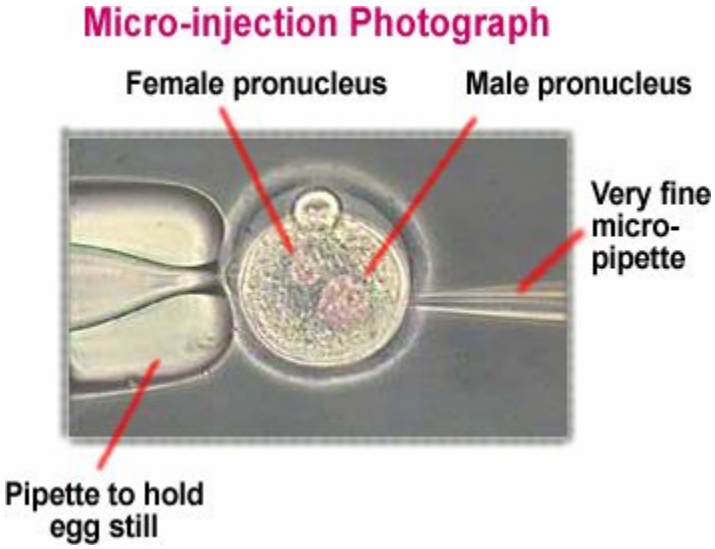

Figure-3: Picture of Microinjection of DNA into the Male Pronucleus. The egg (center) is held in place by a suction pipette (left) while the male pronucleus (pink) is microinjected with a glass pipette (right). This figure was taken from (Genoway…, 2003).

When the pronuclei have eventually fused to form the diploid zygote nucleus, the zygote is then allowed to divide through mitosis into a two-cell embryo. Finally, the two-cell embryos are implanted into a pseudopregnant foster mother (Figure-4) who has been induced to act as a recipient by mating with a vasectomized male in order to create a transgenic animal[2].

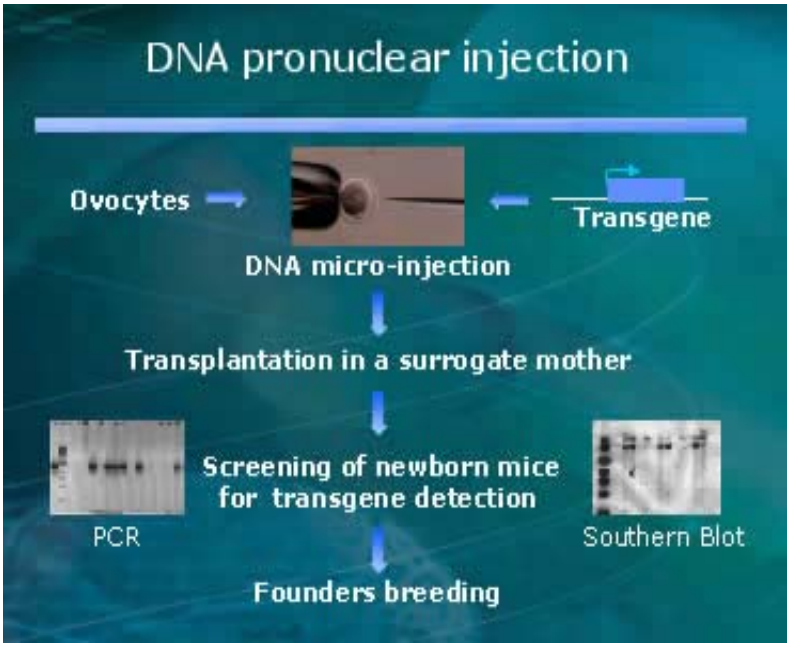

Figure-4: Summary of the Pronuclear Injection Procedure for Making a Transgenic Animal.
Fertilized eggs (upper left) are used for microinjection of the transgene (upper right) and the egg is allowed to divide to either the two cell stage or to the blastocyst. The embryo is then implanted into the uterus of a foster mother (diagram center), and the transgenic pups are screened for the presence of the transgene by PCR or Southern blots (lower portion of the diagram). This figure was taken from (Genoway… 2003).

The Embryonic Stem Cell Method

The second approach, also known as the Embryonic Stem (ES) Cell Method, is also performed by first using recombinant DNA methods to clone the transgene. However, rather than inserting the DNA directly into the male pronucleus, the DNA is applied to cultured ES cells so that they may incorporate it (Figure-5).

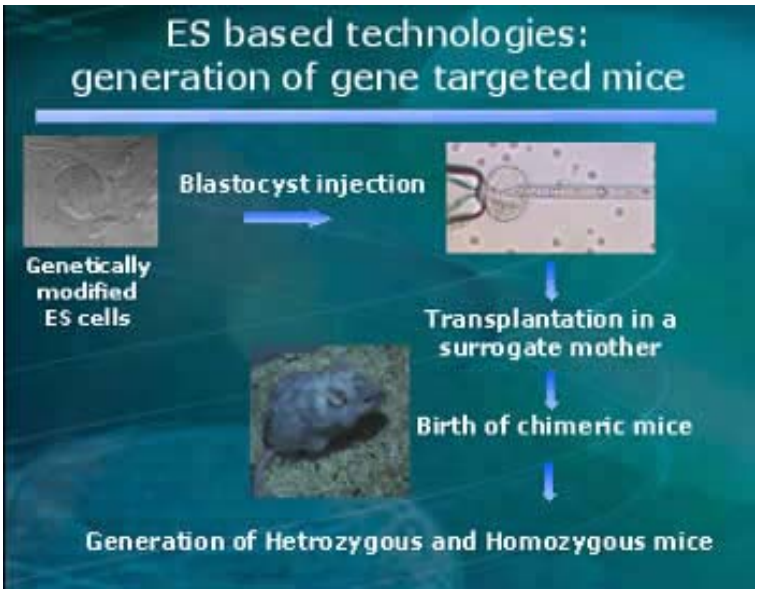

Figure-5: Summary of the ES Method for Making a Transgenic Animal.
Cultured ES cells are transfected with the cloned transgene (upper left), then those cells are injected into a blastocyst (upper right). The blastocyst is implanted into a foster mother to produce transgenic pups. This figure was taken from (Genoway…2003).

Once this process is complete, the successfully transformed cells are chosen and injected into the inner cell mass of blastocysts cultured from that species. Finally, a pseudopregnant animal is prepared by exhibiting the stimulus of mating in order to facilitate the hormonal changes necessary for the animal's uterus to become receptive. The embryos are then transferred

to the uterus with the hope that they will become implanted and eventually yield the transgenic animal (Genoway….2003).

Flaws of the two Methods

While both methods of creating transgenic animals have been successful, neither are without their flaws. The main flaws of the Pronucleus Method are associated with the fact that microinjected DNA inserts at random positions in the host chromosome, so the insertion position of the DNA could have some drastic effects on the outcome due to modification of the pattern and level of expression of the transgene, silencing of the transgene expression, as well as the disregulation of other gene's expression due to the transgene integration site (Genoway..2003). The flaws of the Embryonic Stem Cell Method are due to the difficulty of culturing ES cells, and the low survival of ES-injected blastocysts. Both methods carry the flaw that there is no control over the successful implantation the embryos within the uterus, in fact, tests have demonstrated that no more than one third of the embryos will implant successfully (Transgenic…2003).

Other Approaches to Making a Transgenic Animal

Homologous Recombination

Various modifications exist to these two main approaches for making a transgenic animal. One of these modifications is known as homologous recombination (Figure 6). In this process the transgene is flanked by DNA representing known regions of the host DNA. Once the construct is introduced into an ES cell or male pronucleus, the portions of DNA identical to the

host DNA undergo homologous recombination to replace the cloned sequences with their counterparts in the host chromosome. In this process, the transgene is not only inserted, it is inserted at a known location (Genoway..2003). This homologous recombination approach allows scientists to target a mutation in a specific location of the genome, as well as delete or express certain genes within the genome.

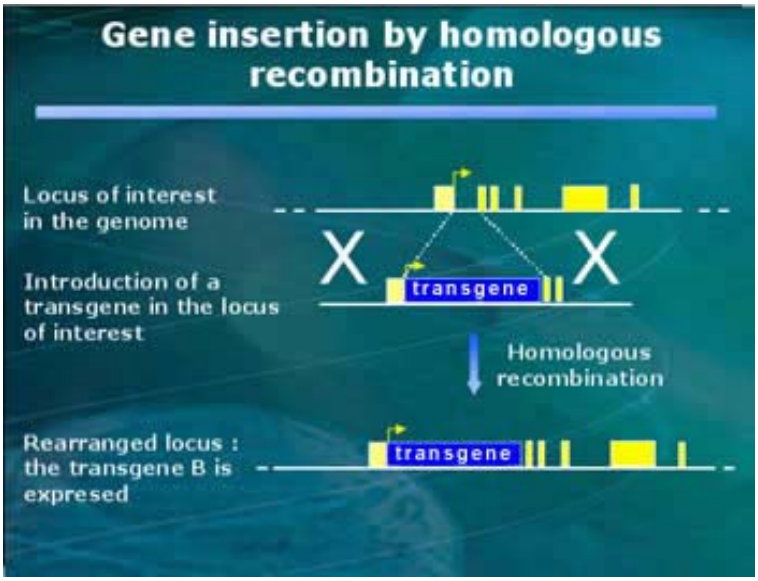

Figure-6: The Homologous Recombination Method for Inserting a Transgene at a Specific Site.
The transgene (blue) is flanked by host chromosomal regions. Once the construct enters the cell, it undergoes homologous recombination (large X's in the diagram) in which the cloned regions replace their counterparts in the host chromosome. This figure was taken from (Genoway..2003).

Viral Delivery

In some cases, viruses are used to deliver the cloned transgene inside the host cells. In this approach, specific deliterious viral genes are removed from the viral DNA, then replaced by the transgene of interest. The DNA is then packaged into virions that are used to infect ES cells. The infection process is quite efficient, and is used to improve gene transfer thus increasing the probability of expression (Transgenic..1997). Viruses are usually used as vectors to transport genetic material into the cell due to their natural ability to infect host cells. The downside to this

approach is that in order for the transgene to be successfully transmitted, the retrovirus must manage to integrate into some of the germ cells.

Chapter 2: Transgenic Animal Classification and Examples

A transgenic animal is any animal that is given a gene it does not normally have. There are many types of transgenic animals, and the purpose of this chapter is to describe some of the animals that have been created so far, and their purposes. There are disease models, like the Oncomouse and the Alzheimer's mouse, whose purpose is to mimic some aspect of human disease to facilitate its study. There are transpharmers, goats that can make human protein and drugs in their milk. There are xenotransplanters, pigs engineered with the hope of transplanting organs from them into humans. Some are intended food sources, such as fish created with extra growth hormone. There are scientific models that can teach us about biological mechanisms and development, like ANDi the transgenic monkey.

Disease Models

Disease models are among the most important transgenic animals. Animals have long been used in medical research to test medications, vaccines and other treatments for some time. Unfortunately, for many human diseases their corollary does not exist in the animal kingdom, so a normal mouse or rat cannot be used as a model. For example, mice simply do not contract Alzheimer's disease. It is impossible to test the effectiveness of a new treatment if your disease model cannot contract the disease (or some key aspect of the disease) in the first place. These special situations have given rise to many important transgenic animals.

Sickle Cell Models

In 1990, Thomas Ryan, et al, developed some transgenic mice that had human sickle hemoglobin. These mice developed a kind of sickle cell disease remarkably like the human version. "Except for their smaller size, sickled erythrocytes from the transgenic mice were indistinguishable from those of the patient with sickle cell disease" (Ryan et al, 1990). The red blood cells in the mice mimicked the physiological behavior of human sickle red blood cells completely. This was one of the first successes in creating a transgenic mouse that could develop a human disease. Ryan went on to note that "although sickle cell disease was the first genetic disorder to be understood at a molecular level, no adequate treatment or cure is available. Hopefully, new drug and gene therapies can be designed and tested in these animals and, therefore, provide new strategies for treating this debilitating disease" (Ryan, et al 1990) .

Alzheimer's Mouse

In 1995, Dr. Dave Adams, et al, made the first true transgenic Alzheimer's mouse. This mouse was engineered with human amyloid genes so it could develop Alzheimer's symptoms. The two main cellular hallmarks of Alzheimer's are amyloid plaques that form extracellularly in the brain, and neurofibrillary tangles that form within the neurons. The large plaques impede brain function. This mouse was the first to show substantial plaque accumulation, and true neurodegeneration (Games, and Adams et al, 1995). The animals were subsequently shown to exhibit behavioral features of Alzheimer's patients, such as memory loss on a maze test.

Since then, there have been many Alzheimer's mouse models created, and many subtle improvements over the original. In 1996, Karen Hsiao et al, developed a mouse with "a fivefold increase in A-beta (1-40) and a 14-fold increase in A-beta (1-42/43)" (Hsiao, et al 1996) which

are protein cleavage products of amyloid. Those amyloid degradation proteins are believed to be the main cause of neurodegeneration. These mice showed normal learning and memory at 3 months, but after 9 months started to show problems. They developed abnormalities very similar to Alzheimer's disease (Hsiao et al, 1996).

In 1999, Shenk et al used the original mouse model created by Prof Adams and colleagues, to create the world's first Alzheimer's vaccine. By injecting a portion of the amyloid protein into the blood of the Alzheimer's mouse, antibodies formed against the plaques, which crossed the blood brain barrier at small lesion sites to clear the plaques out of the brain. The vaccine treatment in young animals prevented plaque formation, while the vaccine treatment in old animals that already had plaques cleared them out. Most importantly, after plaque clearance, the animals performed better on a maize test, showing a regain of brain function. This vaccine is currently in Phase II testing with human Alzheimer's patients with Elan Pharmaecuticals (Shenk, 1999).

Oncomouse

Cancer is currently one of the major killers of humans worldwide. A great deal of research is being done to find better treatments and cures for cancer. One of the most controversial transgenic animals is Oncomouse. Oncomouse is a transgenic mouse containing several human genes that are known to cause cancer. Among these are the genes for neuroblastomas and neuroblastoma leukemia sarcoma virus (N-myc and N-ras). (Leder, 1984) He is an important model for testing new cancer treatments, originally created by Harvard University researcher Phil Leder (Leder, 1984) The Oncomouse line has taught us a great deal

of information about the initial causes of cancer, and became extremely famous as the worlds first patented animal (discussed in a subsequent chapter).

Schizophrenia Model

In 1999, Mohn et al, reported a new transgenic mouse that could prove to be a very good model for schizophrenia. The mouse is genetically engineered to produce only about 5% the normal number of NR1 subunits of the NMDA glutamate receptor, a receptor that is essential in neurological pathways. They found that less NR1 activity results in "increased motor activity and rapid repetitive behavior, as well as reduced social and sexual interactions" (Mohn et al, 1999). These are typical symptoms of schizophrenia. While few treatments currently exist, these mice can help us to better understand the disease, and find new methods of treatment (Mohn et al, 1999) .

Transpharmers

Transpharming involves the use of a genetically modified animal to produce large amounts of a useful product, such as a hormone or protein. Several kinds of transpharming experiments have been performed. Initially, drugs were produced in the blood of transgenic animals. Blood could then be withdrawn and filtered for the desired protein. However, Transpharming an animal's blood has some obvious obstacles, such as the limited amount of blood that can be safely drawn from an animal. Mice have very little blood, and even with larger animals only small amounts of blood can be safely removed. These volume issues caused a change in the focus of transpharming to producing drugs in the milk. (Archer et al, 1994).

In order to produce a milk transpharmer, there were many things that had to first be overcome. First the technique had to be proven successful in animals other than mice. In March of 1991, Lothar Hennighausen's group was able to produce a pig expressing large quantities of whey acidic protein (WAP) which is found in rabbits and small rodents, but is not present in pigs. They gave these pigs the mouse version of the WAP gene. The pigs produced the WAP protein at about 1 gram per liter of milk, levels similar to those found in mice. (Hennishausen, et al, 1990) Hennighausen's group proved that it is possible to produce high levels of a foreign protein in the milk of farm animals (Wall et al, 1991).

Johanna Archer's group was one of the first to successfully produce a human protein in a large transgenic animal. In March of 1994 she wrote, "The concept of mammary gland as a bioreactor has spurred investigation into production of a transgene with mammary specificity, as milk is easily collected from lactating animals" (Archer et al, 1994). Milking a cow or goat is far easier than periodically collecting blood from it. A rather large volume of product can be attained from these animals as well, since they produce so much milk. She goes on to point out that "protein production can reach as high as 1 kg/day in cattle and 200 g/day in goats" (Archer et al, 1994).

Archer was able to express human growth hormone, hGH, in the milk of goats after directly transferring the gene that makes hGH into the goat's mammary gland. They got about 12 ng of hGH in every mL of milk after 15 days. Although this expression level was relatively low, it did prove that farm animals could be used to produce a human transgenic protein (Archer et al, 1994).

A group headed by Angelika Schnieke was able to produce transfarming sheep in December of 1995. These sheep were able to produce human clotting factor IX in their milk.

Human factor IX is the clotting factor that most hemophiliacs lack. Producing this factor in sheep milk could prove easier and cheaper to treat people with hemophilia (Schnieke et al, 1997).

Cattle are ideally a much better animal for transforming since they can produce so much milk. Many obstacles exist before cattle can be made into transpharming animals. One of the largest setbacks is cost. The methods for inserting genes are getting better, but are still very inefficient. Large numbers of treated embryos may only yield a few transgenic animals. Thus working with larger animals, like cattle, is much more expensive than working with mice or pigs. In 1994 Baby Herman was the first transgenic cow ever produced (Biotech Notes, 1994). He was engineered to produce the antimicrobial agent lactoferrin. He was subseaquently mated with normal cows produce female offspring that transpharmed lactoferrin in their milk. In 1998, Jose Cibelli and James Robl were able to make eleven cloned cows. The nuclei from aged donor fibroblast cells containing a Neo^r marker gene were microinjected into enucleated recipient nonquiescent fetal fibroblasts. "Out of 28 embryos transferred to 11 recipient cows, three healthy, identical, transgenic calves were generated" (Cibelli and Robl, 1998). The transgenic calves showed the presence of the marker gene. This was a great success toward creating transgenic cows for transpharming. These methods could be applied and used to make cows with human proteins (Cibelli and Robl, 1998).

The amount of transpharming work done with cows is relatively small, due to their higher cost and longer gestation period, so recent work has focused on goats. Nexia Biotechnologies, a Canadian company, developed and "patented what it calls a breed-early/lactate-early (BELE) goat, which takes up less space than a cow and eats less food" (Willingham, 2000). They developed these goats for their own work in transpharming. Jeffery Turner, President of Nexia,

said “with cows it takes nine months to put a calf on the ground. We went with goats for earlier results. We’ll have goats producing milk in seven months” (Willingham, 2000). Nexia’s transgenic goats have been engineered with a protein for spider silk. They anticipate that their goats can produce about two to fifteen grams of spider silk in each liter of milk. This may not seem like a large amount, but their initial plans call for a herd of 700 goats, with the goal of later having a herd of several thousand. With that many goats Nexia would be able to produce a large amount of the spider silk, which they have patented as BioSteel. They plan on using the silk to make bulletproof vests and similar protection equipment. Bulletproof vests are currently made out of Kevlar, which can be heavy and inflexible. Spider silk is lighter and stronger. Turner said that, “Spider silk’s tensile strength is such that it can withstand a weight of up to 300,000 pounds per square inch” (Willingham, 2000). The silk could also potentially be used in medicine “as stitches, replacement tendons and wiring for prosthetic devices” (Willingham, 2000). These goats could help to develop a whole new line of products in defense and medicine (Willingham, 2000).

Genzyme Transgenics located in Charlton, Massachusetts has a herd of goats that transpharm tPA, a clot busting protein (Ebert, et al, 1991). If used quickly, tPA can minimize the damage from a heart attack or stroke. These animals have a great medical value. The importance of animals like Genzyme’s goats was even noted in the Worcester Telegram and Gazette (Eckelbecker, 2002).

Xenotransplanters

A “xenoplanter” is an animal whose organs can be transplanted into humans. Xenotransplantation is another long term goal of working with transgenic animals. There has

been much work to develop a transgenic pig that can grow a heart suitable for transplantation into humans. These pigs could save countless lives of people awaiting organ transplants. As might be expected there are many obstacles to xenotransplantation. For example, pigs produce a sugar that is present on the surface of its organs. Since, primates do not make this sugar, their immune system sees it as a foreign object and the organ is rejected (Butler, 2002).

On January 2nd of 2002, PPL therapeutics, the same company that first cloned Dolly the sheep, announced the birth of five female knockout piglets. Knockout animals are made without a particular gene. These pigs were created with an inactivated gene for alpha-1,3-galactosyl transferase. This gene encodes the enzyme responsible for adding alpha-1,3-galactosyl on the surface of pig cells. This is the sugar that causes the rejection of pig organs in primates. However, these pigs are not completely without the sugar. Every gene has two copies, or alleles. In many cases only one good copy of the gene is needed for it to be active, as a sort of backup system. Only one of the alleles these piglets have is inactive. The active allele still makes the sugar marker. The hope is that by breeding these pigs with similarly created male pigs, complete knockout piglets can be made. Then the problem of alpha-1,3-galactosyl in pig organs will be solved. These knockout pigs are a step toward viable xenotransplantation (Lai et al, 2002).

David Sachs, director of the Transplantation Biology Research Center at Massachusetts General Hospital, is using another approach to help with xenotransplantation. Declan Butler wrote in *Nature* that, "Sachs is collaborating with Immerge BioTherapeutics on a system where cells from the thymus of the donor pig are first engrafted into the recipient while their immune system is temporarily disabled" (Butler, 2002). While the patient's immune system recovers he will recognize those pig cells as "self." By tricking the patient's immune system the chance of a successful organ donation should rise greatly (Butler, 2002).

Many fears still remain in regards to xenotransplantation. There is a risk that by transplanting organs from animals to humans that we might also transplant infectious agents present in the transplant. Experiments have already shown the occurrence of hepatitis virus infections during some liver transplants. And pigs are well known to incubate various forms of the influenza virus, including strains that infect humans.

Food Sources

Some work has been done to produce transgenic animals that could become an abundant food source.

Superpig

In 1989, Pursel et al, engineered a pig with human growth hormone, hGH. Named Superpig, he grew to an immense size (Pursel, 1989). His creators were hoping to make a larger leaner animal, but while these animals were certainly larger, they developed serious medical problems. The speed at which they put on weight "increased by 15%, feed efficiency by 18%, and carcass fat was reduced by 80%" (Rollin, 1995). But the unexpected problems included "bulging eyes, thickening skin, gastric ulcers, severe synovitis, degenerative joint disease, heart disease of various kinds, nephritis, and pneumonia" (Rollin, 1995). The animals had to be euthanized. Since then the scientific community has placed a moratorium on engineered animals with hGH (Rollin, 1995), and Superpig is often used as a bioethics example of an experiment that should be discontinued.

Superfish

With a rising global population, demand for food is rising. According to the Food and Agriculture Organization of the United Nations, demand for seafood in particular is rising, and may double by the year 2040. Wild fisheries are becoming depleted, so it is likely that there will be more demand placed upon aquaculture. Transgenic fish could potentially offer solutions to these problems (Stokstad 2002).

There have been several fish engineered with extra growth hormone. Cuban biologist Mario Pablo Esrada Garcia of the Center for Genetic Engineering and Biotechnology in Havana altered an African freshwater fish, the tilapia. They gave the fish a viral promoter to increase its own growth hormone. These fish have been observed in the lab to grow twice as fast as domesticated tilapia (Stokstad, 2002). A British group led by Norman Maclean of the University of South Hampton has tested a number of growth enhanced tilapia. They have found that "on average, the transgenic tilapia were three times heavier than nontransgenics at harvest" (Stokstad, 2002). Zhu Zuoyan heads a group in China that has been working on a Yellow River carp with growth hormone from the Grass carp. It has shown 42% faster growth than nontransgenic carp (Stokstad, 2002).

The most famous growth-enhanced fish is probably the modified Atlantic salmon made by Aqua Bounty Farms, Inc. of Waltham, Massachusetts (Devlin, 2001). They added growth hormone from a Chinook salmon along with a promoter. "The modified fish put on weight up to six times as fast as traditional hatchery salmon. Although they don't end up larger than normal farmed Atlantic salmon, they reach market size up to a year sooner" (Stokstad, 2002). This could be a major boom for domesticated fisheries. The modified fish was under review by the FDA in September of 2002. It would need approval from the FDA before the company could

sell fish or eggs to farmers (Stokstad, 2002). But the transgenic fish do not appear to have any of the health problems associated with Superpig.

Scientific Transgenic Models

Working with transgenic animals has led to a great deal of scientific knowledge about the biological effects of over-expressing or under-expressing specific proteins. Before many of these animals could be created, our understanding of genetics and development had to grow. These animals have already taught, and will continue to teach us a great deal. Many transgenic animals have been made as scientific models, to further our understanding of genetics and biology.

ANDi

In 2001, Anthony Chan et al, of the Oregon Regional Primate Research Center, announced the creation of ANDi, the world's first transgenic primate (Chan, et al, 2001). ANDi, whose name represents "inserted DNA", backwards, is a rhesus monkey and carries the jellyfish gene encoding green fluorescent protein. The gene is inactive, but present and detectable in his cells. Of the original 224 eggs that had the gene added, ANDi is the only one to be born with a copy of the gene. The low success rate displayed here can likely be blamed on our lack of experience with in vitro fertilization (IVF) in the rhesus monkey. Ironically, in an article about ANDi published in *Science*, Gretchen Vogel comments "in fact, ethic considerations aside, the project might have been easier to achieve in humans, for whom IVF technology is much more advanced" (Vogel, 2001). The successful creation of ANDi proves that the techniques used to insert foreign genes can work in primates (Vogel, 2001).

Much could be learned from transgenic primates. It is difficult to study many disorders in mice and rats, their physiology is simply too different from humans. Primates have the potential to be much better disease models for aging, behavior, neurological and immune diseases. Gerald Schatten, a member of the group that created ANDi, said that "genetically altered monkeys could be a boon to developmental biologists as well" (Vogel, 2001). He noted that since monkeys are large enough to fit into magnetic resonance imaging machines (MRIs), things like organ development could be tackled with gene markers. He went on to say, "ANDi and his future cousins and brothers and sisters will help us bridge the gap between what we know in the mouse and what we're keenly interested in human development" (Vogel, 2001) ANDi brings us a few steps closer to being able to effectively treat genetic disorders in humans. With the knowledge gained from animals like him we may eventually be able to cure genetic diseases before birth (Vogel, 2001).

Intron Model

Transgenic animals were used to test hypotheses about genetics in 1991. A group led by Richard Palmiter from the Howard Hughes Medical Institute at the University of Washington made some transgenic mice that lacked introns in a modified rat growth hormone gene. Introns are long and seemingly random sequences in DNA that separate the parts known to code for something. The purpose of introns has yet to be fully understood. By making mice with a modified rat growth hormone gene they could study the role of introns. The gene normally has four introns. They made mice that were missing each of those four introns, and some that were missing a combination. Interestingly it seems that only the first intron in the rat growth hormone

gene is essential for hormone production, while the other three are less important. Transgenic animals like these mice can help us unravel many of the mysteries of genetics (Palmiter, 1991).

Doogie the Smartmouse

In 1999, Tang et al, made a transgenic mouse who can teach us much about the way animals, and humans learn. Tang's group made transgenic mice that over-express the NR2B subunit of the NMDA receptor (Tang, et al, 1999). They wanted to study an experimental model of synaptic plasticity known as long term potentiation, or LTP. NMDA is a membrane protein that regulates the initiation of LTP. LTP is believed to contribute to memory and learning. When drugs are used to block it, rats can no longer find their way out a maze, etc. The mice created by Tang, et al, display an enhanced level of LTP, and improved recognition memory. They were tested in a variety of different tasks and displayed a better memory. It has been difficult to reach a consensus in the scientific community on the exact role of synaptic plasticity in learning, but we can learn much from mice like the ones Tang et al, created (Bliss, 1999).

Other Transgenic Animals

Transgenic animals have also been created that do not fall nicely into any of the preceding categories. These are the stranger instances of transgenic animals. For example, a company in the United States is reportedly seeking to develop an interesting breed of transgenic cat. They want to isolate and remove the genes in the cat that commonly cause allergies. A hypoallergenic pet is hardly a worthy focus of transgenic animal research. But since work is allegedly being done to create them, it seems worthy of mention (Anon, 2001). In any case, the

purpose of this chapter was to introduce the reader to the types of transgenic animals that have been made, and their purposes, to allow a discussion about their ethics in the following chapter.

Chapter 3: Transgenic Ethics

Many kinds of transgenic animals have already been made, and the technology exists to create many more. However, there are ethical issues to be considered when creating any transgenic animal. Ethics have long played a role in the biological scientific community. Bernard Rollin wrote in an article about genetic engineering in animals, "the subject matter studied by science is determined by social ethical values" (Rollin, 1996). The ethics of a society deem what areas are worthy of study. For example, ethics places more value on a cure for AIDS than cure for baldness. Rollin continues, "When biomedical research is performed on rats rather than on unwanted children, and the control of pain in these rats is socially mandated, the method of science is determined by social ethical values" (Rollin, 1996). The ethics of society further mandate how research is conducted. With the subject matter and the methods both dictated by the ethics of a society, Rollin concludes, "the very logic of science is modulated by social ethical concerns" (Rollin, 1996).

Ethics must always be considered in any branch of science, and transgenic animals have garnered a lot of attention from ethicists. One negative generalization about biotechnology, and genetic engineering specifically, is that the scientists are "playing God" and altering nature. Rollin argues "If 'playing God' in this area is intrinsically wrong, it is hard to see why damming rivers, eradicating smallpox, and building cities is not also wrong" (Rollin, 1996). Humanity has evolved to its current state by manipulating the environment. We have built settlements, planted farms, raised animals and cured diseases. All of these things altered nature, but are not themselves intrinsically wrong. Similarly, genetic engineering, in and of itself, is not intrinsically wrong (Rollin, 1996).

Jeremy Rifkin claimed that genetic engineering "desacralizes nature" and wrongly "violates species integrity" and "crosses species barriers" (Rifkin, 1985). Rollin counters that argument by explaining that "species are not the fixed, immutable rigid building blocks of nature that Aristotle and the Bible believed them to be" (Rollin, 1996). Evolution exists, and species change over time. Humanity has already played a major part in the evolution of animals and plants. "It is estimated that 70% of present-day grasses and 40% of flowering plants were 'created' through human artifice, and vast number of animals have been drastically modified (domestic animals for example)" (Rollin, 1996). Humans have long been playing a major part in the evolutionary process. The biotechnology that allows us to create transgenic animals alters individual members of a species, but can greatly speed up the "evolution" process (Rollin, 1996).

Particular classifications of animals have been hotly debated, along with individual transgenic animals themselves. Each general classification of transgenic animals comes along with its own set of concerns. The issues over many of these general groups should be addressed before specific animals are discussed. Thus, it is the opinion of this author that transgenic experiments should ethically be considered on a case-by-case basis.

Transgenic Disease Model Ethics

Disease models are perhaps the most debated classification of transgenic animals. They offer society great proven and potential medical benefits, by way of treatments and cures for some of the worst diseases plaguing mankind. However, in order for them to help us discover new methods of treatment, we must give them the human disease, or portions of the disease. In some cases, like Alzheimer's mice, because no other models exist, the animals are absolutely required for moving forward with the human vaccine that Elan Pharmaceuticals is currently

working on (Schenk et al, 1999). These animals can teach us much about how to treat the disease, with little or no apparent suffering (they learn slower on a maze test). So this author feels that the benefits to society greatly outweigh any negatives for this line of experimentation.

In other cases, like Oncomouse, the potential medical knowledge is great about what causes cancer formation, and how to treat it, but the animals can clearly suffer (they develop tumors). Oncomouse is so controversial that in 2002 Erika Check reported in *Nature* that the Canadian Supreme court refused the patent. The potential research uses for this mouse that develops cancer was so great that Harvard applied for a very far reaching patent. Harvard applied for the patent in the United States**,** Europe, Japan and Canada. Canada was the only country to deny the patent. The Canadian Supreme Court acknowledged that the method of making the mouse may be patented, but they denied Harvard the right to patent the mouse they created. In its ruling the court said, "A higher life form is not patentable because it is not a 'manufacture' or 'composition of matter'" (Check, 2002). Canada is not a major part of the global biotechnology market, so the decision was not seen as a major setback to Harvard. But this ruling shows just how controversial transgenic animals can become (Check, 2002).

In the same article, Jo Dufay, campaign director of Greenpeace Canada said "We think the court got it right. These issues are so complex that they require full public debate, and that goes beyond a simple tinkering with the Canadian patent act" (Check, 2002). Patenting life is major issue with transgenic animals and will be fully addressed in the next chapter. But at the same time this is a serious ethical concern. Research and development costs for new drugs are extraordinary, as are the costs of creating animals like Oncomouse. In order for companies to continue pushing research in these areas, they need to be able to make a profit. They need to be

able to fund the research to create the next animal that could help find the next cure for a disease (Check, 2002).

Animal testing is essential for disease treatment. Whenever a new medication is developed, it needs to be tested for possible side effects and drug interactions. Some of these effects may only happen in a small percentage of the population. In order for the drug manufacturer to be confident that all the side effects are explored, they need to run studies with several thousand individuals. It would be extremely difficult to find thousands of human volunteers to test brand new medications. The animal testing in these situations is vital for the development of the new drug. This situation does not change for drug testing in transgenic animals. This phase of testing is just as vital when transgenic disease models are involved. For the case of Oncomouse, due to the enormous amount of scientific information that mouse line has provided on the mechanisms of oncogenesis, this author feels that specific transgenic experimentation should be continued, provided that all efforts are made to minimize animal suffering, by either sacrificing the animal prior to tumor maturation, or by using pain killers.

Transpharming Ethics

Transpharming animals are an interesting classification of transgenic animals. These animals have been modified to produce useful proteins in their milk. Clotting factors have been made in sheep's milk to help treat hemophiliacs. Transgenic goats have been made that produce silk fibers in their milk. These fibers have been extracted and used to make material for a new type of bulletproof vest. The potential uses for this technology are widespread and varied (Willingham, 2000).

Cows are likely the most promising animals to be used as transpharmers because they produce large amounts of milk, and they have a long lifespan compared to mice or goats. However, there are more than financial considerations to creating transgenic cows. Hindus believe that cows are sacred animals. Do we have the right to tamper with sacred animals? Hindus treat cattle with great reverence and respect. The animals provide them with an abundance of resources. They burn the manure for fuel, and they drink the milk for food. While manipulating the genetics of a cow may be tampering with a sacred animal in the eyes of a Hindu, not all of us are Hindus, and much is at stake medically. If a cow can make important antibodies and proteins in its milk, then it could help save human lives. Since Hindus drink the milk from cows, it seems reasonable for us to make a cow with life saving proteins in its milk. This would make the animal even more important, as it would be providing more than simple nourishment for the people using the products in the milk.

Because hundreds of cow embryos may be needed to make one successful transgenic cow, we must also consider how damaging is the loss of life to a culture that views the cow as sacred? The important aspects to consider with transpharming animals are the potential gain against potential suffering. Transpharming animals have a lot to offer to society. They can potentially be efficient ways to produce life saving proteins. These animals can make clotting factors for patients with hemophilia, or produce other medications. And importantly, these animals suffer no more than domestic animals that are being milked.

Therein lays the issues with transformers. Domesticated cows in the western world are given hormones so they produce more milk. They are also milked by machines for long periods of time. The animals can become very uncomfortable; udders can get sore and swollen. If the milk is produced from a transpharming cow, it will be more valuable than the milk of a normal

cow. It is likely that transpharming animals will be pushed at least as hard to make more of their valuable milk. So in this authors view, transpharming experiments should continue since the positives (huge medical benefits) strongly outweigh any negatives (there is no animal suffering, although embryo losses are high during the cloning process).

Xenotransplantation Ethics

Xenotransplantation is a major concern for many activist groups. They fear that with the transfer of organs from animals to humans there will also be transfer of diseases. This fear is one reason why non-human primates are not often used. The Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) published in September of 1999, "It now seems likely that non-human primates will not be used as tissue or organ donors in New Zealand" (ANZCCART, 1999). They do not want to risk an HIV like epidemic and also believe "a relatively slow reproducing animal that is close to being an endangered species should not be used as a tissue donor" (ANZCCART, 1999). Monkeys are in some ways too similar to humans to use as tissue donors. Their diseases are often very similar, so the risk of diseases jumping to humans is much higher (ANZCCART, 1999).

Surprisingly, pigs are the more widely accepted animal donors to humans. They are similar enough that pig heart valves and skin have already been successfully used in transplants. "[Pig] pancreatic islets have been used in New Zealand in the treatment of type II diabetic patients" (ANZCCART, 1999). However, much work needs to be done before entire organs can be considered for transplantation, because the pig organs are often rejected by the host's immune system. The risks are legitimate, but the incredible medical gains that could be achieved with xenotransplantation urges research to continue (ANZCCART, 1999).

The U.S. Public Health Service issued guidelines for xenotransplantation in 2000. Soon after the release of these guidelines, Brian Carnell wrote an article to address many of the activist's concerns. He first stressed the incredible gains xenotransplantation has to offer, stating "13 people in the United States die every day while waiting for an organ transplant, and any advance that utilized animal tissues or organs would save many lives" (Carnell, 2000).

The Campaign for Responsible Transplantation does not believe the 2000 guidelines were adequate because it is impossible for xenotransplantation to be risk free. The concern of diseases transferring from animal to human is legitimate; after all we have witnessed such diseases jumping species since humans first domesticated animals. Influenza first infected humans through pigs, and continues to transfer between humans, pigs, and birds. The scientific community is currently following the outbreak of H5N1 bird flu in China for fear of its evolving into a strain that readily infects humans. In addition, "in vitro research has demonstrated that retrovirus carried by pigs can infect human cell lines" (Carnell, 2000). Viruses tend to be very resourceful "organisms". They can adapt to a new population very quickly. This means that jumping species may not be overly difficult for a disease. Although most scientists admit the concerns are legitimate, "the risk is not great enough to forego the advantages of this technology" (Carnell, 2000). We simply must take proper steps to reduce the risks (Carnell, 2000), for example like raising the pigs in viral free environments.

No medical procedure will ever be completely risk free. Risks will always be part of the medical field. No treatment will work 100% of the time. The best course is to minimize the potential risks. Animals to be used for xenotransplantation should be as free of disease as possible. Carnell writes, "animals intended for xenotransplantation use will be special breeding

populations that are kept under special clean laboratory conditions" (Carnell, 2000). This would be the best way to avoid animal diseases.

Using pigs to cure humans with diabetes would generally be considered a good thing, but the Campaign for Responsible Transplantation wants to consider the cost in animal life. "Up to 100 pig fetuses may be needed for a single transplantation of pig pancreatic islet cells into a diabetic patient. Each patient may need several transplants during the course of treatment. That's a lot of pigs for one person" (Campaign for Responsible Transplantation, 2000). Current transgenic technology is rather inefficient. Many animals are sacrificed for these treatments. Activist groups will force us to keep an eye on the costs of treatments. But based on past experience, usually the more a given procedure is used and modified, the more efficient it becomes, so this could reduce the number of required animals in the future. This author feels that this line of transgenic experimentation should be continued, with special attention paid to raising the animals in "clean" laboratory conditions.

Ethics of Transgenic Food Sources

Transgenic animals have been made to serve as a better food source. Genetically modified foods have been on the market for some time. With the technology to create transgenic animals, food sources can further be modified. But this will, of course, come with more controversy.

Superpig is widely regarded in the scientific community as a mistake. Superpig was made with the gene for human growth hormone. The hope was that he would grow bigger, leaner, and faster than other pigs. He did grow very large, very quickly, but developed health problems, from arthritis to various organ failures, that he had to be euthanized. This author

agrees this line of experimentation should be discontinued, since there is no strong medical benefit, and considerable animal suffering.

There have been several transgenic fish made with extra growth hormone. Most have growth hormones from other kinds of fish in addition to their own. These fish do not suffer as superpig did, and they grow much faster. Fish that are adapted to the waters of many developing nations have been modified. The African freshwater fish, the tilapia, has been modified by both Cuban and British groups. The British group reported that "on average, the transgenic tilapia were three times heavier than non-transgenics at harvest" (Stokstad, 2002). A group in China has modified a yellow carp with the growth hormone from grass carp, and reported the modified fish are 42% bigger than regular yellow carp. One of the better known varieties is a modified Atlantic salmon made by Aqua Bounty Farms, Inc. of Waltham, Massachusetts. This fish can grow up to six times as fast as a normal salmon (Stokstad, 2002; Devlin, 2001).

These fish can potentially be used to help fight hunger in the developing world since they can reach market size so much faster, and they could feed a large population in a short period of time. With a rising global population, demand for food is rising. According to the Food and Agriculture Organization of the United Nations, demand for seafood is rising, and may double by the year 2040. Wild fisheries are becoming depleted, so it is likely that there will be more demand placed upon aquaculture. Transgenic fish could potentially offer solutions to these problems, giving large populations food relatively cheaply (Stokstad, 2002).

When using genetically altered food in undeveloped nations there is a problem of dependence. These fish will out produce native fish, and local fish farmer would need to raise the transgenic fish to compete. However, if for some reason these fish do not bred as well as normal, the poor nations may need to continuously buy eggs from foreign companies. The

modifications may also unknowingly make the fish more susceptible to disease, creating further problems for the developing nations.

An obvious risk with these transgenic fish is their ability to grossly out compete their native counterparts. If fish somehow got out of the fisheries then the local area would undoubtedly be overrun with them. Great precautions should be taken to insure that these modified fish do not get into the local habitats, as they could certainly wipe out the natives. Destroying the local environment can be very damaging.

Ethics of Transgenic Scientific Models

Transgenic animals made as scientific models can provide a lot of knowledge to the world community. These animals help expand our knowledge of genetics and biology. This better understanding makes it possible for countless advances in biology and medicine in the future. In many cases it is also possible to create these animals with no suffering on their part.

Such is the case with ANDi, the world's first transgenic monkey. He was made by Chan, et al, of the Oregon Regional Primate Research Center. He contains a copy of a jellyfish gene that can be easily assayed. The green fluorescent protein gene is present in his cells, but is not expressed for some reason. ANDi proved that the methods for creating him were sound, and he opens up the possibilities for creating other transgenic monkeys in the future. Primates would make much better models for many human diseases. Before those disease models can be made, the methods need to be perfected for primate transgenesis (Vogel, 2001).

The smart mouse made by Tang, et al, in 1999 is another scientific model that teaches us something significant while not inflicting any suffering upon the animal. The mouse over-expresses the NR2b subunit of the NMDA receptor, and he shows improved recognition memory

(Tang, et al, 1999). The mouse in no way suffers by being able to recognize things better. He does teach us more about how memory works, leading the way for other advancements. The main argument against creating scientific models is the tampering with life, mentioned previously, which is involved in making any transgenic animal (Bliss, 1999).

A concern with any transgenic animal is the low success rate. Hundreds of embryos may be needed to get one viable pregnancy, especially for large farm animals. Out of several pregnancies, few will survive to actually be born. This is seen by many to be a great waste of animal life. If the animal offers little to society once it is created, then this is a great waste of animal life. If the animal has much to offer in terms of better medical and scientific advancement, then the loss of life in the initial creation of the animal must be considered, but eventually accepted when large numbers of human lives are at stake.

Some people and groups are wholly against transgenic animals because of the genetic engineering involved. The technology is not completely understood. Bernard Rollin wrote, "it is impossible to effect simple one-to-one correspondence between gene transfer and the appearance of desired traits" (Rollin, 1996). Segments of DNA often have more than one effect. Many experiments with creating transgenic animals have caused unexpected results. For example, some mice were engineered to produce more interleukin 4, which is important to the immune system. The hope was to use these mice to study different aspects of the immune system, but the mice developed osteoporosis (Lewis et al, 1993). Genetics is not completely understood, and may never be. For this reason, the creation of transgenic animals should always be done with great care (Rollin, 1996).

Animal rights advocates denounce what they deem as frivolous experimentation on animals. However, most people will agree that in order to make medications to cure diseases

like cancer and Alzheimer's disease some testing on animals is needed. They will rightfully get upset in those situations if the animals are treated cruelly. Sacrificing an animal's life is far more justified if it is helping to cure a disease, than perfecting a new makeup line.

There is a priority that develops from these realizations. Those animals that will have the greatest benefit on society, by say curing cancer, should be made and used. Those transgenic animals that will have the least positive effect on society should probably not be made. Research into non-life-threatening experiments, like exactly which gene in cats creates the allergen that people react to, can wait until more pressing questions are answered.

Simply because we posses the technology to create a transgenic animal, does not mean that the animal should be made. The pros and cons of creating a specific animal must be considered before it is created. The potential contribution to human society be it through disease research, a path to a greater food supply, or general advancement of knowledge must be considered alongside the negative impacts of creating the animal. The most obvious negatives include any suffering the animal may go through and the general tampering with and loss of life involved in creating these animals. To impose undue suffering upon an animal is unethical. To tamper with life simply as an exercise of our own power is also unethical. Before a transgenic animal is created its potential gains to society should be considered. If there is very little to be gained by creating such an animal it should not be made, regardless of the amount of suffering to it. Likewise, if an animal potentially has a great deal to offer, then it should be made with every attempt made to minimize its suffering.

Often the researchers involved with creating and using these animals have the utmost respect for them. A transgenic mouse that helps to develop a cure for a disease deserves some credit. It is safe to assume that the researchers working with these animals know their worth to

society, and will treat them humanely in accordance with their university's IACUC animal care codes. Aside from the respect they should have for such a valuable tool in their research, is the expense of the animal. Transgenic animals are not created easily or cheaply. They are valuable. The animals should be respected by the people that work with them, at least as much as the expensive equipment in the laboratory.

Transgenic Ethics Conclusions

The Alzheimer's mouse (Games et al, 1995) can offer a great deal to the medical community. Normal mice simply cannot get the disease, but these allow us to test and develop treatments. These mice also do not suffer, and seem perfectly happy by mouse standards. They are frisky and play with other mice. They eat, sleep, and breed quite normally. It is clear that Alzheimer's mouse was a good transgenic animal to make.

Transpharming animals also approach this ideal. Goats and cows can produce life saving proteins in their milk, without appearing to suffer. Cows produce more milk than their calves often need, and it can be painful for them to not be milked. These animals do not mind being milked by humans, and there is no apparent suffering involved in producing extra proteins in that milk. Thus, Transpharmers appear to also have a large amount of gain with little suffering. They are another animal that should be made.

If the potential gain of a transgenic animal is minimal, but the suffering great, then it clearly should not be made. Such was the case with superpig. Superpig was engineered with a gene for human growth hormone. The intent was to produce a bigger pig with leaner meat. The extra growth hormone had many unforeseen effects however. Then superpig became very obese, and developed crippling arthritis in its legs. As other organs threatened to fail, superpig was

euthanized. Here the suffering of the animal clearly outweighed any potential gain. Pigs are not especially hard to come by, and breed happily on their own. This animal clearly should not have been made.

Some transgenic animals have been made for more trivial reasons, like making a more enjoyable pet. Animals that offer no real betterment of human life should also not be made. There has also been work on creating a transgenic cat that does not produce common allergens. This is another unnecessary animal, for slightly different reasons than superpig. The cats may not suffer at all, but their potential contribution to society is so small. If someone is allergic to cats, they should simply not have one as a pet. They should not seek out a modified cat that is missing the gene for the allergens. This animal also serves little purpose in helping the world community and should not be made (Anon, 2001).

But what if the potential gain from a transgenic animal is great, but so is its suffering? How then should it be decided what is a good enough reason for an animal to suffer. No animal should be made to suffer for petty reasons. These are the cases that spawn the most controversy. Oncomouse is and will likely remain one of the most hotly debated transgenic animals. Oncomouse is vital for the development and testing of new cancer treatments. But the mouse does develop tumors, which is what the treatments are tested on. These tumors undoubtedly cause pain and suffering to the animals. But the medical benefits of cancer treatments cannot be ignored.

Oncomouse was and is a breakthrough in cancer research. He makes it possible for new and better cancer treatments to be developed. As better treatments are developed, more cancer survivors will have been saved, at least in part, by oncomouse. The knowledge to be gained from him is incredibly valuable, but oncomouse can clearly suffer.

With oncomouse it is possible to test treatments before the tumors become advanced enough to cause pain. Also pain medications can be applied. This middle ground is helps answer the ethical question of oncomouse. He has an immense value to the medical and scientific community, and his suffering must be minimized whenever possible. This is a good way to achieve both of those goals.

Before creating a transgenic animal, both sides of the issue must be considered. The potential gains of such an animal must be compared with the suffering that it will incur. If it does not suffer and can be a great benefit to society, then it should be made. Conversely, if the animal suffers greatly with minimal benefit to society, then it should not be made. The difficulty comes with the animals that both benefit society and suffer. In these cases it is necessary to minimize the animals suffering. If the suffering of the animal can be sufficiently reduced then it is the opinion of this author that the animal should be made. If the suffering of the animal cannot be reduced, the animal should not be made. Extreme suffering should not be imposed upon any animal, even with great potential gains.

Chapter 4: Transgenic Legalities

Along with the new era of science involving the creation of transgenic animals came the inevitable question "should animals be patented?" This question brought a large amount of controversy. The US Constitution reserves for the federal government the power to grant exclusive patents (Edwards, 2001). In 1973 the Congress passed the Patent Act which identifies the three elements needed for a patent. First, the invention must be useful, second, the invention must be novel, and finally, the invention must not be obvious (Edwards, 2001).

Patents are used by companies or individuals to restrict competition. The patent system has become a significant economic incentive for the production and proliferation of animals used in medical and other forms of research, and will provide a source of income to enable more such animals to be created, and discourage the use of alternatives. A person or organization can now challenge newly-issued patents through both the U.S. Patent and Trademark Office and the federal court system. With the coming of the twentieth century, Congress began to modify the patent laws in order to account for humanity's new ability to alter and even create plant life. In 1930, Congress passed the Plant Patent Act which allowed for patents on newly-discovered or invented plants. The first idea of an actual animal patent came in 1988 when the Patent and Trademark Office issued its first animal patent to the transgenic mouse known as Harvard Mouse (Guidelines..2005).

The Oncomouse Case

In America, the Harvard Mouse (also known as Oncomouse which had been developed at Harvard Medical School through Dupont funding) (Figure-7), became the first patented animal in 1988. "Onco" is derived from the word Oncogene or oncogenesis, referring to the form of human cancer the mouse develops over time (No patents..1997). Based on previous experiments identifying *myc* as an oncogene whose mutations cause human cancer, this oncogene was incorporated into a mouse line so the altered gene sequence would be inheritable. Since the Oncogene was inheritable this mouse along with all of its offspring would prove extremely useful in cancer research due to the fact that the mouse would be prone to develop cancer in a fairly predictable way.

Figure-7: A Picture of Harvard and Dupont's Oncomouse. This figure was taken from (Thompson , 2002).

In the process of issuing the patent to Harvard University for the Oncomouse, the US became the first country in the world to issue a patent for an animal. It is interesting that later that year Oncomouse was labeled as "Product of the Year" by a popular financial magazine. Controversy over this topic was so strong that legislation for a moratorium on animal patents was

considered by Congress in 1987 and 1989. Later an animal protection organization even tried to file a legal challenge, however, the attempt was unsuccessful. Since then, the Oncomouse has gone on to be patented in both Europe and Japan.

As a result of the Oncomouse case, three US patents were passed, US4736866: Transgenic non-human mammals, which covered the method for creating transgenic non-human eukaryotic animals whose germ cells and somatic cells contain an activated oncogene sequence introduced into the animal, or an ancestor of the animal, at an embryonic stage. Also, US5087571: Method for providing a cell culture from a transgenic non-human mammal, which covered the method for creating cell lines derived from transgenic animals. Finally, US5925803: Testing method using transgenic mice expressing an oncogene, which covered the methods used to assay transgene expression in transgenic tissues or cell lines (Mitchell and Somerville, 2002).

The Oncomouse Case in Canada

In 1995, a 15 year-long case involving the infamous Oncomouse began in Canada. When Harvard attempted to patent the Oncomouse, the Canadian Commissioner of Patents denied the patent declaring that the living creature was not an invention within the meaning of the Canada Patents Act. After hearing the decision, Harvard appealed to the trial division of the federal court where Judge Nadon upheld the decision of the Commissioner of Patents stating "on even the broadest interpretation I cannot find that a mouse is "raw material" which was given new qualities from the inventor. Certainly the presence of the myc gene is new, but the mouse is not new, nor is it "raw material" in the ordinary sense of that phrase…A complex life form does not fit within the current parameters of the Patent Act without stretching the meaning of the words to

their breaking point, which I am not prepared to do" (Stop…2005). Still Harvard would not give up, in August 2000 Harvard appealed Judge Nelson's decision in the Federal Court of Appeal. In a split 2-1 decision the Court allowed Harvard a patent on the mouse itself.

This decision however, inspired still more controversy. On August 20, 2000 Canada's Quakers wrote to the Prime Minister and begged the federal government to appeal the decision of the Federal Court of Appeal due to the fact that animals were all God's creation. Several groups of environmental activists also challenged the decision of the Federal Court of Appeal claiming that the animal's right to life was being violated by the patent.

On October 2, 2000, the Government of Canada filed an application to appeal the Oncomouse decision to the Supreme Court of Canada, shortly after this, the Canadian Council of Churches along with the Evangelical Fellowship of Canada obtained intervener status in the case. The churches invited lawyer William J. Sammon who pointed out that when the Canada Patent Act was passed in 1869, the framers of the legislation had no idea that the Act would eventually be used to patent an animal (Stop…2005). As a result , on December 5, 2002 the Canadian Supreme Court issued its oncomouse decision: "the sole question in this appeal is whether the words manufacture" and "composition of matter", within the context of the Patent Act, are sufficiently broad to include higher life forms. It is irrelevant whether this Court believes that higher life forms such as the oncomouse ought to be patentable" (Stop…2005). Thus, in the end the Supreme Court of Canada ruled 5-4 against Harvard University, declaring that mice and other "higher" animals could not be patented according to Canadian laws (No patents..1997). Even Harvard's argument that genetically-altered animals should be legally defined as "patentable machines," was no use, and Canada refused to patent the Oncomouse. The Court decision stated that, "Several important features possessed by animals distinguish them from

both micro-organisms and plants and remove them even further from being considered a 'composition of matter' or a 'manufacture.' In particular, the capacity to display emotion and complexity of reaction and to direct behavior in a manner that is not predictable as stimulus and response, is unique to animal forms of life" (Stop…2005). In spite of the Court's denial of the patent for Oncomouse, it did allow for patent claims to be placed on the process used to create the genetically-altered mice. The Supreme Court had based its no-patent ruling only on the meaning of the existing Canada Patents Act, however, the judges noted that Canadians must think about the issue much more broadly through their parliament. Mr. Justice Bastarache declared "this Court does not possess the institutional competence to deal with issues of this complexity, which presumably will require Parliament to engage in public debate, a balancing of competing social interests, and intricate legislative drafting" (Stop…2005).

Other Patented Animals

Since 1987, over 460 patents have been granted on animals in the United Sates. A few of these patents on animals include one which claims beagle dogs who have been exposed to radiation in order to virtually destroy their immune systems and whose lung lobes are infected with a particular species of mold. According to the patent team, the dogs can then be used to test various drugs and to learn more about the mechanisms of this type of infection. Another patent claims cats, non-human primates, sheep, pigs, goats, cattle, and dogs whose healthy hearts were surgically altered to mimic a "fatal human infant condition" called transposition of the great arteries (TGA) and then immediately surgically "corrected," thus resulting in unusual circulatory connections to mimic "congenitally corrected TGA." The patented animals are used in surgical training and TGA-related experiments and research (Edwards, 2001).

Opinions on Animal Patenting

Various animal rights organizations are strongly against animal patenting. Some of these organizations include the WSPA (World Society for the Protection of Animals), PETA (People for the Ethical Treatment of Animals), and the SACA (Students Against Cruelty to Animals) (Subject…2004). All of these organizations believe that the patent clearly violates the animal's right to life and to its own body. They believe that animals being kept by or under the control of humans must be kept in circumstances that are appropriate to their species. They also believe that animal suffering is inherent in these patents since most involve directly harming or altering animals for research, testing, and experimentation (Mitchell and Somerville, 2002).

When it comes to the matter of patenting animals I believe that the benefits gained from the patents outweigh the negatives. I believe that animals do have rights, and I do not approve of animal cruelty, but I believe the potential pain can be managed humanely to minimize suffering while still allowing the medical benefit to society. While the patenting of an animal may infringe upon the animals rights, there are too many benefits to be ignored. The patented animals allow research which otherwise could not be performed, as in the case with Oncomouse. I believe that animal patents are necessary in order to gather the full benefit of transgenic animals.

Benefits of Animal Patenting

Some of the benefits of animal patenting include the fact that transgenic animals and human gene sequences have enormous commercial value in agriculture, biomedical research, medicine, and the pharmaceutical industry. The impact of these forms of

biotechnology on society is astounding. In addition to providing accurate and cost-effective models for the study of human disease, transgenic animals are capable of improving food sources and disease resistance in animals (Guidelines..2005). Through the use of transgenic technology it is also possible to engineer animals to produce pharmaceutical products in their milk, or even organs that are capable of being transplanted into humans.

Transgenic animals have made researching causes and possible treatments of disease so easy that they have been called a "gold mines for researchers" (Guidelines..2005). Mice, such as the Oncomouse are used as living laboratories in order to remove significant amounts of the guesswork from toxicological studies. The method by which scientists remove this guesswork involves "color-coding" the genes they insert into a mouse. The color shows up whenever a mutation occurs, thereby signaling the harmful nature of a chemical. Transgenic animals are able to allow a quick check for a color change in germ cells to signal mutation, rather than using thousands of laboratory subjects which would be necessary for similar testing. Transgenic animals enable studying of the first-generation animal as well as its subsequent generations, thus enabling researchers to observe the effects of the genetic mutations in the transgenic animal's offspring. The results of studies with transgenic animals are quicker, less expensive, and more realistic than previous methods (Guidelines..2005).

Transgenic animals also improve the quality of disease study in many cases; in fact, in some cases transgenic animals are the only way to study a particular disease. Some substances which are harmful to humans do not appear as harmful in animal subjects. Through the use of transgenic animals, serious human sicknesses are able to be studied without the use of any human subjects. Currently transgenic-animal disease models exist

for AIDS, sickle cell anemia, Down's syndrome, hepatitis B, Alzheimer's disease, high cholesterol, and various cancers (Guidelines..2005). Taking transgenic animals even further, scientists hope that they will become the source of donor organs in the future. Researchers have already managed to successfully transplant human organ tissue into mice, and hope that the use of organs such as livers, kidneys, or hearts of animals will be available in the future for those in need of transplants. Finally, by developing transgenic animals as disease models, pharmaceutical companies have a more economic and realistic way to test their products. Pharmaceutical companies may test the effectiveness of vaccines and drugs by creating the animals to be prone to certain sicknesses. Transgenic animals will be very helpful in the actual production of pharmaceuticals as well.

The pharmaceutical industry uses both transgenic animals as well as human gene sequences in order to help aid people with sicknesses such as genetic disorders, hormone deficiencies, and enzyme deficiencies. By altering the DNA of some animals, it is possible to create animals which secrete beneficial proteins in their milk. An example of one such animal includes a transgenic goat which could produce a drug used in the treatment of cystic fibrosis in its milk. A transgenic sheep also exists which is able to produce up to five ounces a day of a protein used to treat emphysema (Guidelines..2005). Insulin, human growth hormone, and drugs for the treatment of heart attack and stroke victims are all possible candidates for future production in the milk of transgenic animals.

Animals have been used for the purpose of producing proteins such as human growth hormone and insulin for a long time; however, the animals were previously sacrificed in order to obtain the protein. With today’s transgenic technology, it will no longer be necessary to sacrifice the animals in order to produce the proteins necessary for

various pharmaceuticals. The new transgenic technology will also allow for easier and far more cost effective mass production of the proteins. Since transgenic animals are able to produce proteins far more economically than common methods, it is estimated that the current cost of such proteins will be reduced by as much as 100 times (Guidelines..2005). Finally, the quality of the drugs produced with transgenic animals and gene sequences may be much higher than drugs produced synthetically.

Pitfalls of Animal Patenting

Some of the potential pitfalls of patenting animals include the fact that it could actually hinder research. Researchers in the field warn that patents in science promote secrecy prior to the granting of a patent. They also hinder the free exchange of ideas and information necessary for co-operative scientific effort. In other cases, research organizations, both public and private, which have been the first to isolate a gene may restrict the terms under which other scientists continue to work with that gene (Thompson, 2002). For poorer countries in the Third World, the impacts of patenting are likely to be especially severe. As Piet Bukman put it when he was responsible for development cooperation at the Dutch Ministry of Foreign Affairs: "A fence is being built around biotechnological know-how, which can only be opened from the inside" (Thompson, 2002). And it is the developed countries which hold the key.

The patents could also lead to restriction of the research agenda. Scientists are concerned that as the research agenda becomes increasingly commercialized, a process greatly enhanced by patents, funds will be channeled into what is commercially

profitable rather than being used for the public good (Thompson, 2002). It is disturbing that, as far as human genetic disorders are concerned, the quest for patentable products is likely to divert much-needed funding from research into preventative health measures that would benefit the public but bring few profits to biotechnology corporations. “It has been estimated, for example, that at least 90 per cent of human breast cancers are unrelated to breast cancer genes but are triggered by environmental pollutants, diet and lifestyle factors” (Thompson, 2002).

The patents can also restrict competition. It turns out that patent law not only permits monopoly control of new technologies and processes, but also actively endorses such powers. Far from encouraging competition and in turn leading to new research, patents actually inhibit it. This is especially true for patents covering a wide array of inventions. In medicine, companies holding patents are often being given a complete monopoly over the development of all protein products derived from the gene (Thompson, 2002). The access to treatment is also restricted. Patented treatments are more likely to sell to high income patients rather than those of a lower income. As a result, it can also be determined that what is good for business may not always be beneficial for the patient.

Such patents can be used to exploit publicly-funded research. The leaping advances in transgenic knowledge over the past 40 years are largely due to publicly-funded education and research, financed with money from the taxpayer or charitable bodies. The public will thus have to pay the price of research twice over if patents are granted on genetic "discoveries". To make matters worse, public financing will have to continue if the transgenic industry is to survive. "The publicized vigor and successes of transgenic companies may foster illusions that basic research can be left to industry,"

points out Kornberg. In the US: "More than 90 per cent of such research has, in the past, and must be, in the future, done in university and other academic settings, requiring massive support to the tune of billions of dollars from the taxpayer through the federal government" (Thompson, 2002). In this academic situation, not only does the transgenic industry benefit from the taxpayer's money, but it also benefits from the good nature and co-operation of society.

Conclusions of Animal Patenting

After presenting both the pros and the cons of animal patenting it can be concluded that animal patenting is extremely beneficial to society as long as the animals are treated as humanely as possible and the transgenic research is used for the public good. If these steps are taken many of the cons of animal patenting will be eliminated, leaving many pros to outweigh the remaining cons. Thus, the pros of animal patenting can outweigh the cons as long as a few steps are taken to ensure that the patenting is done in a protective manner and for a just cause.

CONCLUSIONS

Transgenic animals may be defined as animals that have foreign genes inserted into their DNA in order to cause them to exhibit traits or characteristics not normally found in their species. In the ongoing quest of transgenic research, two main approaches have been used to create these transgenic animals. One approach involves manipulating the male pronucleus, while the other involves manipulating ES cells. Each method poses individual advantages and disadvantages, unfortunately neither of the two methods are particularly efficient for large farm animals.

Transgenic animals are sometimes used to help study various aspects of human health. Since an animal's DNA can now be altered to make it susceptible to a human disease that its species does not contract under natural circumstances, scientists are able to use these animals as model systems for human diseases and thereby provide a system for testing new treatments and medications that is not possible with human subjects. Some of the diseases for which transgenic animals have served as model systems include Alzheimer's Disease, Sickle Cell Anemia, and various forms of human cancers.

Transpharming involves the use of a genetically modified animal to produce large amounts of a useful product, such as a hormone or protein. Successfully transpharmed proteins include human growth factor, clotting factors, emphysema drugs, and clot busters. These desired proteins are usually produced in the animals milk, where it can easily be harvested without sacrificing the animal, and in fact their behavior indicates the animals likely are not even aware they are manufacturing the protein, so they don't appear to suffer. Cows seem to be the most

promising animals to be used as transpharmers because they produce large amounts of milk, and they have a long lifespan compared to mice or goats.

Xenotransplantation involves the modification of animals to produce organs that can be transplanted into humans, while at the same time minimizing immunorejection. Xenotransplantation is more of a long term goal of working with transgenic animals, and scientists hope to be able to have organs ready for emergency organ transplants in the near future. One recent example involves the construction of a transgenic pig lacking cell surface sugars viewed as foreign by the human immune system. These Xenoplanters have the potential to save the lives of countless people who die awaiting organ transplants, however, special attention must be paid to the possibility of viral contamination of the organs since they are transplanted from a different species, and viruses can sometimes jump species.

Transgenic animals have also been made to serve as scientific models for research, and to broaden human understanding. By over or under-expressing certain proteins, much can be learned about the role of specific proteins on how different biological systems function and develop. Many human disorders and diseases would be better modeled on transgenic primates than transgenic mice, however, it is important that the scientific community first understands more about creating higher order transgenic animals since this has not been done to the same extent as mice for example.

Along with the progression of science, the continual concern of human ethics has always played a large role in society. For the controversial technology of transgenics, once again ethics strongly influences the outcome of scientific research. Simply because transgenic technology that allows us to create an animal exists does not necessarily mean that we have the right to take advantage of that technology. Current protocols in transgenic science require very careful

consideration before any animal is created. These protocols state that if the benefits to society are great and there is no apparent suffering for the animal, as with Alzheimer's mouse or transpharmers, then the creation of the animal is justified. And the authors of this IQP agree with this stance. However, if there is little benefit to society and the animal suffers greatly, as with Superpig, then the creation of the animal cannot be justified. In certain cases where there are both great benefits and substantial suffering for the animal, it is necessary to find a balance between minimizing the potential suffering of the animal and maximizing the potential benefit to humanity, only when this has been accomplished do we feel the transgenic animal should be created.

Along with the ethical dilemmas associated with the creation of transgenic animals came the inevitable question of "should animals be patented?" The first idea of an actual animal patent came in 1988 when the Patent and Trademark Office issued its first animal patent to the transgenic mouse known as Harvard Mouse. In the process of issuing the patent to Harvard University for the Oncomouse, the United States became the first country to issue a patent for an animal, arguing the procedure used to create oncomouse was useful, novel, and not obvious based on prior art.

However, patenting life is a controversial topic. Several animal rights groups including WSPA (World Society for the Protection of Animals), PETA (People for the Ethical Treatment of Animals), and SACA (Students Against Cruelty to Animals) oppose the patenting of animals because they believe that the animals rights to life are being violated (Subject…2004). Feelings against animal patenting were so strong that in Canada even the oncomouse patent was eventually denied by its Supreme Court, arguing patent laws do not apply to animals. Through these few cases it is obvious that the controversy surrounding animal patenting will not end in

the near future. Despite this, after the oncomouse patent succeeded in America it went on to be approved in Europe and Japan. Since the oncomouse case, over 460 animal patents have been approved in the United States alone.

The technology used to create transgenic animals has opened new doors to the scientific community and demonstrates amazing potential to save human life. This technology will enable scientists to find new disease treatments, new ways of making medications, and even whole new ways of looking at organ transplantation. With this technology comes a myriad of ethical and legal concerns that must be considered with care. It is the opinion of the authors of this IQP that transgenic technology used legally and ethically can greatly improve the quality of life for humanity, but oversight committees must continually be vigilant to enforce tight controls over ensuring minimal animal suffering.

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